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ORIGINAL ARTICLE

Liver stiffness (Fibroscan®) is a predictor of all-cause mortality in people with non-alcoholic fatty liver disease

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Abstract

Background and aims: Progressive liver fibrosis related to non-alcoholic fatty liver disease (NAFLD) is associated with all-cause and liver-related mortality. We assessed vibration-controlled transient elastography (VCTE) as a predictor of mortality.

Method: Data from patients who underwent VCTE for NAFLD at four large health services in Victoria, Australia between the years 2008 and 2019 were linked to state-wide data registries. Cause of death (COD) and predictors of all-cause mortality were subsequently analysed using descriptive statistics and Cox-proportional regression analysis.

Results: Of 7079 VCTE records submitted for data linkage, 6341 were matched via data registry linkage. There were 217 deaths over a 22 653 person-year follow-up. COD included malignancies other than hepatocellular carcinoma (HCC) (18.0%, $n = 39$), sepsis (16.1%, $n = 35$), decompensated liver disease (15.2%, $n = 33$), cardiac disease (15.2%, $n = 33$) and HCC 6.0% ($n = 13$). Controlled attenuation parameter (CAP) was not associated with mortality in univariable analysis (HR = 1.00, CI 1.0–1.0, $p = .488$). Increased liver stiffness measurement (LSM) (HR 1.02 per kiloPascal, CI 1.01–1.03, $p < .001$), Charlson comorbidity index (CCI) (HR 1.32 for each point, CI 1.27–1.38, $p < .001$) and age (HR 1.05 per annum, CI 1.03–1.07, $p < .001$) were each associated with higher rates of all-cause mortality in multivariable analysis. LSM ≥10 kPa suggestive of compensated advanced chronic liver disease (cACLD) was associated with mortality in multivariable analysis (HR 2.31, CI 1.73–3.09, $p < .001$).

Conclusion: VCTE LSM, in addition to age and CCI, is independently associated with increased all-cause mortality in a large cohort with NAFLD.

KEYWORDS
cause of death, Charlson comorbidity index, elasticity imaging techniques, non-alcoholic fatty liver disease, registries

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; cACLD, compensated advanced chronic liver disease; CAP, controlled attenuation parameter; CCI, Charlson comorbidity index; CKD, chronic kidney disease; COD, cause of death; CSPH, clinically significant portal hypertension; CVD, cardiovascular disease; db/m, decibels/metre; HBV, hepatitis B Virus; HCC, hepatocellular carcinoma; HCV, hepatitis C Virus; HIV, human immunodeficiency virus; HR, hazard ratio; IQR, interquartile range; kPa, kiloPascals; LSM, liver stiffness measurement; MAFLD, metabolic-dysfunction associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; NIT, non-invasive tests; T2DM, type 2 diabetes; VAED, Victorian Admitted Episodes Dataset; VCTE, vibration-controlled transient elastography; VDI, Victorian death index.

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent disorder that is estimated to affect 25.4% of the global population.[1] NAFLD is associated with a swathe of extra-hepatic conditions including cardiovascular disease (CVD), renal disease and gastrointestinal neoplasia including hepatocellular carcinoma (HCC) which are thought to be driven by heightened systemic inflammatory and profibrotic pathways.[2,3]

Progressive liver fibrosis stage is associated with increased liver-related and overall mortality in NAFLD.[4–8] Progressive fibrosis is frequently asymptomatic until such time that complications of advanced disease manifest. It is hence of paramount importance to identify and stage NAFLD at a population level in order to determine those who are most at-risk for adverse outcomes and to introduce timely interventions that prevent liver and extrahepatic disease complications.[9]

There is, in addition, an unmet need for an efficient and cost-effective method of risk stratification of NAFLD.[10] The current gold standard for NAFLD staging is via liver biopsy histological assessment. However, liver biopsy is not practical as a screening test due to cost and potential serious adverse events.[11] An emerging suite of non-invasive tests, some of which are more scalable than others, have therefore been developed as surrogates for liver histology. These include predictive serum-based algorithms, ultrasound-based elastography, magnetic resonance spectroscopy and magnetic resonance elastography.

Vibration-controlled transient elastography (VCTE) is a widely used non-invasive point-of-care test that provides a liver stiffness measurement (LSM) and also a measure of hepatic steatosis via the controlled attenuation parameter (CAP).[12] VCTE has excellent diagnostic accuracy and inter-user reliability for detection of fibrosis, and furthermore delineates mild-to-severe fibrosis from cirrhosis, with a sensitivity of 75% and a specificity of 81.2%.[13,14] Like other non-invasive tests (NITs), VCTE is most powerful in its negative predictive value and can rule out advanced fibrosis in 94.7% of cases.[13] VCTE has been recommended by current clinical guidelines as a tool for risk stratification of people with suspected NAFLD and concurrent metabolic risk factors, such as obesity or T2DM,[10,15] and has an increasing role in stratifying compensated advanced chronic liver disease (cACLD) and predicting clinically significant portal hypertension (CSPH).[16]

Several studies have assessed VCTE as a predictor of mortality, and more recently, as a predictor of cardiovascular, liver and oncological morbidity.[17–19] However, there is no guidelines-based recommendation for use of VCTE as a prognostication tool for mortality. This reflects in part the paucity of large cohort, real-world data evaluating VCTE as a prognostic tool. We, therefore, evaluated the capability of single time-point VCTE to predict mortality in a large cohort of Australian patients with NAFLD. We used linkage analysis to evaluate outcomes and comorbidity data, and in doing so, were able to adjust for the Charlson Comorbidity Index (CCI) which has been widely validated as a predictor of mortality following hospitalisation.[20,21]

Lay Summary

Fatty liver disease is a very common condition which increases the risk of both liver and non-liver health issues. Notably, health conditions external to the liver, including heart disease, account for the majority of deaths in the context of fatty liver disease. Fatty liver disease may have no symptoms until complications develop. Tools, like elastography (including Fibroscan®), which measure the severity of liver damage without the need for biopsy, are important in determining disease prognosis. Our data show that increased liver stiffness measurement based on Fibroscan®, a common point-of-care liver staging tool, is associated with a higher risk of death, even after accounting for many other medical variables. Fibroscan® may therefore be useful in predicting all-cause mortality.

2 | PATIENTS AND METHODS

2.1 | Cohort selection

A retrospective cohort linkage study was conducted. The study was approved by the Monash Health Human Research and Ethics Committee and was conducted in accordance with the Australian National Statement on Ethical Conduct in Human Research. Cohort selection was derived using VCTE data from adult subjects (>18 years) who underwent FibroScan® (Echosens) at four Victorian tertiary referral centres with a combined catchment size of 4.26 million people, between July 2008 and April 2019. Patients with a primary indication of NAFLD, NASH, steatosis or fatty liver disease were included. Patients were excluded if non-NAFLD conditions, such as excess alcohol, viral hepatitis, autoimmune liver disease, other heritable liver conditions or previous liver transplant were recorded in the VCTE study indication. Notably, metabolic-dysfunction associated fatty liver disease (MAFLD) was described following our initial cohort recruitment.[22] At the time of data collection, VCTE services were offered almost exclusively at tertiary health services and could be accessed through medical practitioner referral without preceding specialist Hepatology review, and without prerequisite evidence of intermediate- or high-risk fibrosis based on serum-based NITs.

2.2 | VCTE study evaluation

All non-invasive liver assessments were performed using VCTE according to the manufacturer's recommendations. Subject demographic and VCTE results were collected. VCTE data included the test indication, total elastograms acquired, number of successful elastogram acquisitions, LSM expressed in kiloPascals (kPa), LSM interquartile range (IQR), CAP in decibels per metre (db/m) and CAP IQR. LSM and CAP results were expressed as the median value of

successful measurements. VCTE studies were defined as valid if there were >10 successful acquisitions and if the LSM IQR was ≤30% for studies with an LSM ≥7.1 kPa (Boursier's criteria).[23] In the case of serial or consecutive studies, the earliest valid study was included.

A rule of five for LSM assessed by VCTE (10, 15, 20 and 25 kPa) was used for analysis based on recent Baveno VII guidelines highlighting the prognostic utility of these thresholds in predicting compensated and decompensated liver disease, regardless of aetiology.[16] The CAP score became available at two sites from the year 2012 and at a third site from the year 2017. CAP thresholds of 302, 331 and 337 db/m were used to delineate steatosis grade ≥S1, ≥S2 and S3 respectively.[24] Further sub-categorisation of steatosis ≥S1 was appraised using a CAP threshold of 274 db/m, which has a higher sensitivity than 302 db/m (90% vs. 80%) but lower specificity (60% vs. 83%).[24]

2.3 | Data validation

An a priori decision was made to validate 5% of the total cohort for NAFLD. This target was exceeded, with $n = 445$ prospectively acquired case records from the largest contributing site evaluated for co-existing liver conditions/risk factors, including excess alcohol (>30 g daily for men and >20 g daily for women), blood-borne viruses and other immune-related or inherited conditions. Additional data including, smoking history, body mass index (BMI), serum alanine aminotransferase (ALT) values, and relevant imaging were evaluated as part of this dataset. The validation set remained incorporated within the primary linked cohort.

2.4 | Data linkage

The VCTE dataset was linked to Victorian Death Index (VDI) and the Victorian Admitted Episodes Dataset (VAED) via the Centre for Victorian Data Linkage (CVDL). The CVDL is an accredited data integration agency that generates and maintains linkages with health, clinical and administrative data custodians using de-identified data matrixes. The CVDL matches individuals to central databases via the Victorian Linkage Map using imputation of pre-specified combinations of patient names (using an 85% probabilistic threshold), hospital identification numbers, Medicare® numbers, gender, and/or date of birth as derived from source records. Follow-up was censored at the time of death, liver transplant or at June 2019.

2.5 | COD data analysis

Cause of death (COD) was ascertained from a combination of VDI and VAED data. VDI data, which is derived from Victorian death certificates, provides hierarchical descriptions of primary and associated causes of death. The VAED, which records all public and private hospital admissions, provides a minimum set of demographic data and health-related data which is recorded using International Classification of Diseases 10th Revision (ICD-10) coding. VDI COD for individual patients was compared to VAED ICD-10 coding for admitted episodes in which the recorded separation mode was death. A synthesised COD was reported for each patient after a combined appraisal of VDI and VAED mortality data by a Hepatologist. In cases where there were intersecting causes of death, such as infection as a precipitant of decompensated liver disease, the organ/system failure resulting in death was reported as the primary cause of death, rather than the precipitant. Sepsis was considered to be a standalone diagnosis in cases where there was insufficient attributable evidence of precipitating underlying organ dysfunction or overt secondary decompensation of a chronic condition.

2.6 | Comorbidity data analysis

ICD-10 coded data was extracted from the VAED and used to analyse comorbidities within the linked cohort. The Charlson Comorbidity Index (CCI) was calculated from VAED-linked data using pre-specified codes.[25,26] CCI data was censored within 6 months of the VCTE study in order to calculate 'baseline' comorbidity, with a view to adjusting for CCI in multivariable analysis of mortality determinants.

2.7 | Statistical analysis

Descriptive statistics were used to quantitively define frequencies of observations, including demographics and COD. The percentage of observed events was based on the overall study population denominator, unless stated otherwise. Kaplan–Meier survival curves were generated using LSM thresholds based on the rule of five cut-offs.[16] Categorical variables were compared using the chi-squared test. Univariable Cox regression analysis was used to test for associations between age, gender, country of birth, comorbidity data, and the CCI, LSM and CAP scores. Age, CCI, LSM and CAP were analysed as continuous variables the univariable analysis. A multivariable model, with and without competing risk regression accounting for non-liver-related deaths, was fitted adjusting for univariable data with $p < .01$ as well as age and gender. CCI was used as a surrogate for each individual comorbidity in the multivariable analysis. A two-sided $p < .05$ was used to indicate statistical significance. All analysis was conducted in Stata/BE 17 (Texas, USA, 2017).

3 | RESULTS

3.1 | Study population

There were 13 958 VCTE studies conducted between July 2008 and April 2019 that met the pre-specified inclusion criteria. After selecting the first representative VCTE study and removing duplicates,

7079 individual records remained (Figure 1). A sub-cohort analysis of $n = 934$ individuals showed that 51.6% ($n = 482$) of referrals were from public gastroenterology clinics, 44.0% ($n = 411$) from private gastroenterologists and 4.4% ($n = 41$) from General Practitioners and non-Hepatology specialist outpatient services.

3.2 | Cohort demographics, clinical characteristics and VCTE

The demographic and clinical characteristics of the study population (Table 1) were determined by VAED linkage data. Data extrapolated from each of the four individual sites are shown in Table S1. Median age at VCTE across the 7079-patient cohort was 60 years (IQR 49–69), median LSM was 6.1 kPa (IQR 4.7–9.3) and median controlled attenuation parameter (CAP) ($n = 3789$) was 293 db/m (IQR 240–339). Pre-specified (Boursier's) VCTE reliability criteria were met in 97.5% (6899/7079) of cases.[23] LSM ≥10 kPa, suggestive of cACLD, was found in 22.9% (1624/7079) of VCTE studies, with 12.4% (880/7079) presumptive cACLD due to LSM > 15 kPa. Steatosis ≥S1 steatosis based on CAP ≥274 db/m was recorded in 59.8% (2265/3786) of the cohort. Overseas country of birth was proportionally higher than the national average (43.1% vs. 29.7% respectively),[27] and there was a greater representation of females within the cohort (53.1%). Type 2 diabetes (T2DM), followed by malignancy, and chronic kidney disease (CKD) were the most common comorbidities (19.1%, 5.6% and 3.7% respectively) based on ICD-10 coding from hospital admission episodes ($n = 5858$) (Table 1). Comorbidities were more common in individuals with >1-day admissions. For example, diabetes was coded in 24.6% (760/3084) patients with a >1-day admission, and in 12.9% (359/2774) of those with a <1-day admission. Admissions <1-day were predominantly elective admissions with some overnight admissions through the Emergency Department.

3.3 | Validation set

Case records from 445 individuals within the study cohort were analysed as part of the validation dataset. A NAFLD diagnosis was verified in 88.3% (393/445). In the patients who did not meet NAFLD criteria, 26 individuals consumed alcohol in excess, 6 had a prior history of significant alcohol misuse, 7 had a documented history of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and 13 had other conditions, including sarcoidosis, primary biliary cholangitis, primary sclerosing cholangitis, Wilson's disease, haemochromatosis, alpha-1-antitrypsin deficiency, congestive hepatopathy and non-cirrhotic portal hypertension (Figure 1). In terms of ICD-10 coded 'baseline' comorbidity, 26.2% ($n = 119$) had T2DM, 85.7%

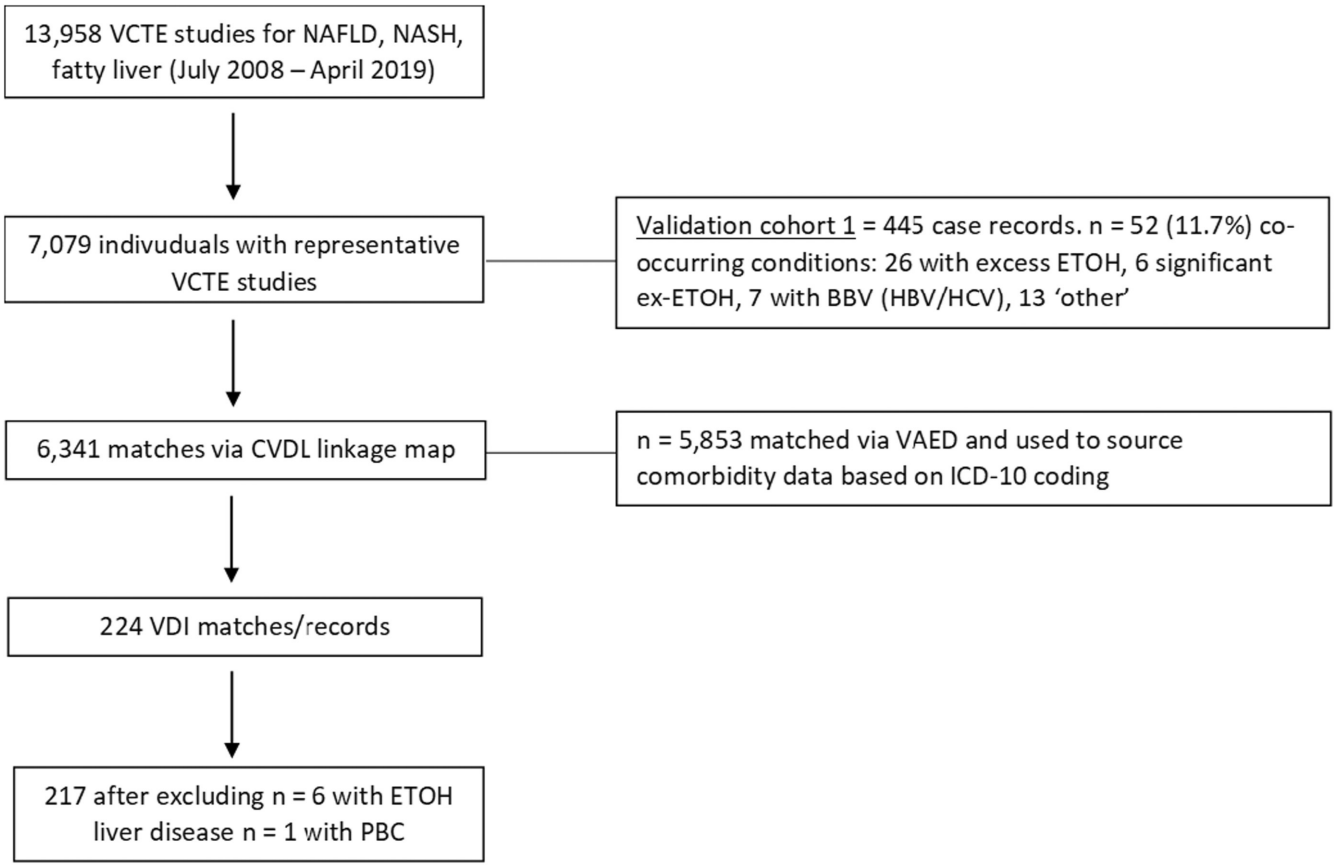

FIGURE 1 Study flow chart. CVDL, centre for Victorian Data Linkage; ETOH, alcohol; HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cholangitis; VAED, Victorian admitted episodes dataset; VCTE, vibration-controlled elastography; VDI, Victorian Death Index.

TABLE 1 Demographics of population ($n = 7079$)

Parameter	*n* (%)
Age, median (IQR)	60 (IQR 49–69)
Age at death ($n = 217$), median (IQR)	72.3 (64.4–79.0)
Gender, $n = 6001$ (%)	
Male	2813 (46.9)
Female	3188 (53.1)
Country/region of birth ($n = 4655$)	
Australia	2650 (56.9)
Europe	913 (19.6)
Asia (Southeast/east/central)	512 (11.0)
Polynesia (New Zealand, Fiji, Cook Islands, Samoa)	111 (2.4)
Indonesia, Malaysia, Philippines	135 (2.9)
Middle East	140 (3.0)
Africa	91 (2.0)
South and central America	71 (1.5)
North American	30 (.6)
Charlson comorbidities VAED ($n = 5858$)[a]	Pre-VCTE[27]
T2DM	1121 (19.1)
Liver disease	660 (11.3)
Cancer	326 (5.6)
CKD	218 (3.7)
Peripheral vascular disease	169 (2.9)
Congestive cardiac failure	159 (2.7)
Chronic ischaemic heart disease	146 (2.5)
Pulmonary disease	134 (2.3)
Stroke	98 (1.7)
Peptic ulcer disease	96 (1.6)
Connective tissue/rheumatological	52 (.9)
Hemiplegia	44 (.8)
HIV/AIDS	10 (.1)
Dementia	1 (.0)
BMI, median (IQR) ($n = 2, 126$)	30.6 (27.0–35.1)
Smoker ($n = 1095$)	
Yes (current/former)	482 (44.0)
Never	613 (56.0)
ALT, median (IQR) ($n = 1047$)	41 (23–65)
CAP ($n = 3786$), median (IQR)[b,25]	293 (240–339)
<302 db/m	2063 (54.5)
302–336 db/m	715 (18.9)
CAP ≥337 db/m	1008 (26.6)
LSM, median (IQR)	6.1 (4.7–9.4)
LSM category, $n = 7079$[16]	
<5 kpa	2183 (30.8)
5–10 kpa	3272 (46.2)
10–15 kpa	739 (10.4)
15–20 kpa	275 (3.9)
20–25 kpa	206 (2.9)
>25 kPa	404 (5.7)

[a]Comorbidity data censored within 6-months of VCTE.

[b]CAP ≥274 db/m, which has a 90% sensitivity for detecting steatosis, was present in 59.8% (2265/3786).

($n = 390$) were obese (BMI ≥30 kg/m^2) and 24.0% ($n = 109$) were prescribed a statin therapy. Portal hypertension based on radiological finding was documented in 1.3% ($n = 6$) of the validation cohort, none of whom had documented decompensated liver disease. Of the people who did not meet NAFLD, criteria validation cohort, 86.5% ($n = 45$) would have met MAFLD criteria based on body mass index (BMI) >25 kg/m^2 and/or a diagnosis of T2DM.[30] VCTE data from the validation cohort are shown in Table S2.

3.4 | Data linkage

Of the 7079 individual records submitted for linkage analysis, 89.6% (6341/7079) were matched to one or more state-wide databases via the CVDL. The vast majority of the linked cohort, 92.3% (5853/6341), had an admission episode captured in the VAED database. Many of the admitted episodes, 47.4% (2774/5853), were <1 day admissions. There was a 22 653 person-year follow-up from VCTE to death/censorship (median 3.6 years, IQR 2.1–5.5, range .2–11.0).

3.5 | Cause of death

There were 223 deaths (3.5%) in the linked cohort over the follow-up period of 657.8 patient-years. Alcohol excess and PBC were attributable to six deaths and were excluded from subsequent analysis, yielding a COD cohort of $n = 217$. The median time to death was 2.4 years (IQR 1.2–4.4, range .1–10.8 years). Median age at death was 72.3 years (IQR 64.4–79.0). Non-HCC malignancy ($n = 39$, LSM 12.3 kPa, IQR 6.0–22.8), followed by sepsis ($n = 35$, LSM 12.0 kPa, IQR 5.8–21.7), decompensated liver disease ($n = 33$, LSM 32.2 kPa, IQR 18.8–49.1) and cardiac disease ($n = 33$, LSM 9.6 kPa, IQR 6.9–21.5) were the most common primary COD, accounting for 18.0%, 16.1%, 15.2% and 15.2% of deaths respectively (Table 2). Sepsis largely occurred in the context of ancillary medical comorbidities, and in 54.3% (19/35) of cases, was due to lower respiratory tract infection and aspiration. Of the 35 patients who died from sepsis, 60.0% had suggestive cACLD based on LSM ≥10 kPa, 57.1% had T2DM, 40.0% had malignancy and 22.9% had CKD stage ≥3. T2DM was the most common comorbidity in people who died during the follow-up period, affecting 50.7% ($n = 110$) of the deceased cohort (Table 3). HCC was a primary ($n = 13$) or comorbid ($n = 6$) COD in 8.8% ($n = 19$, LSM 22.8 kPa, IQR 11.7–31.5) of the deceased cohort. A breakdown of malignancies is shown in Table S3.

TABLE 2 Primary COD based on consolidated death (VDI) and admissions (VAED) datasets

Cause of death (COD)	*n* (%)	LSM (IQR)
Infection[a]	35 (16.1)	12.0 (5.8–21.7)
Decompensated liver disease[b]	33 (15.2)	32.2 (18.8–49.1)
HCC	13 (6.0)	22.3 (12.0–26.3)
Non-HCC malignancy		
Non-GI malignancy	25 (11.5)	13.9 (6.2–23.9)
Non-HCC GI malignancy	14 (6.5)	7.5 (5.9–14.0)
Cardiac complications		
Decompensated cardiac failure	19 (8.8)	9.6 (7.0–21.2)
Myocardial infarction	14 (6.5)	13.0 (7.2–24.5)
Stroke	12 (5.5)	10.8 (8.1–22.6)
Respiratory	9 (4.1)	14.7 (10.0–23.5)
Kidney injury (acute/chronic)	10 (4.6)	8.8 (5.0–24.1)
Vascular	10 (4.6)	13.8 (9.8–21.1)
Other[c]	17 (7.8)	6.9 (4.9–21.6)
Nil documented COD	6 (2.8)	12.7 (7.1–30.9)

[a]Considered causal in cases where infection could not be clearly delineated as either caused by or resulting in comorbid system failure.
[b]Co-morbid HCC in two of these cases.
[c]Includes post-operative complication, MVA, self-harm, neuromuscular/neurodegenerative disorders, GI bleeding.

3.6 | Factors associated with mortality

3.6.1 | Univariable analysis

In univariable analysis, advancing age (HR 1.08 per year, CI 10.07–1.10, $p < .001$), male gender (HR 1.35, CI 1.03–1.77, .028) and each component of the CCI, with the exception of mild liver disease, were associated with increased mortality risk (Table 3). Dementia and HIV were not included in the analysis as there were no deaths recorded in people with these comorbidities. The CCI as a continuous composite variable was also associated with increased mortality risk (HR 1.44, CI 1.41–1.49, $p < .001$) (Table 3 and Figure 2). Increased LSM on VCTE as a continuous variable was associated with heightened mortality in univariable analysis (HR 1.04 per kPa, CI 1.04–1.05, $p < .001$) (Table 3). CAP, as a continuous variable, was not associated with mortality (HR 1.00 per db/m, CI 1.0–1.0, $p = .488$). Higher threshold CAP cut-offs of either ≥302 db/m ($\chi^2 = 2.93$, $p = .087$) or CAP ≥331 db/m ($\chi^2 = 1.18$, $p = .276$), correlating with grade 1 (S1) and grade 3 (S3) steatosis, respectively,[24] were also not associated with increased mortality.

3.6.2 | Multivariable analysis

In multivariable analysis, increased LSM as a continuous variable (HR 1.02 per kPa, CI 1.01–1.03, $p < .001$), increased CCI as a continuous variable (HR 1.32, CI 1.27–1.38, $p < .001$) and advanced age (HR 1.05 per year, CI 1.03–1.07, $p < .001$), but not gender, were associated with increased mortality (Table 3). LSM remained a significant predictor of mortality even after liver disease was excluded from the CCI scoring algorithm (HR 1.03 per kPa, CI 1.02–1.03, $p < .001$), and also after excluding unreliable VCTE studies (Table S4).[23]

3.6.3 | Competing risk regression analysis

A competing multivariable risk regression model was performed. Failure was defined as a composite of death due to decompensated liver disease and HCC ($n = 43$), and competing events were defined as death from other causes ($n = 173$). In this model, the sub-distribution ratio (SHR) for liver-related death was 1.05 (CI 1.03–1.06, $p < .001$) per kPa increase in LSM, 1.17 (CI 1.04–1.31, p .01) for CCI as a continuous variable, and 1.07 (CI 1.03–1.10, $p < .001$) per year of advancing age.

3.6.4 | Prediction of survival using liver stiffness measurement cut-off values

Increased LSM threshold values had an inverse correlation with survival (Figure 3A,B and Tables S5 and S6). In univariable analysis, an LSM ≥10 kPa[16] was associated with higher mortality HR = 5.48 (CI 4.15–7.24, $p < .001$). This threshold value remained significant, HR 2.31 (CI 1.73–3.09, $p < .001$) in multivariable analysis after factoring in age, gender and CCI. The mortality effect size was more notable as LSM threshold values increased. For example, at the time of censorship, 15.5% (82/528) of people with an LSM ≥20 kPa had died, compared to 2.1% (135/6334) people with an LSM < 20 kPa. The survival gap between people with LSM ≥20 kPa versus LSM < 20 kPa widened over time as shown using 1, 3 and 5-year outcomes (Table S6).

4 | DISCUSSION

4.1 | Main findings

Progressive fibrosis stage has been borne out as an independent predictor of all-cause and liver-related mortality[28] across different liver staging modalities, including histopathology, serum-based fibrosis biomarkers (including the NAFLD Fibrosis Score, FIB-4 and aminotransferase to platelet ratio index), and ultrasound-based elastography.[6,8,10,29] To our knowledge this is the largest cross-sectional study to demonstrate an association between increased mortality and liver stiffness on VCTE in the context of NAFLD. Our data show that mortality risk occurs along a continuum of liver stiffness, but is not associated with increased CAP. We show that an LSM ≥10 kPa, suggestive of cACLD, is a predictor of heightened mortality. COD in our study was most frequently due to

TABLE 3 Cox-regression univariable and multivariable analysis of mortality risk ($n = 5857$)

	Univariable analysis			Multivariable analysis[a]		
Parameter	HR	CI	*p*-value	HR	CI	*p*-value
Age	1.09	1.08–1.10	<.001	1.05	1.03–1.07	<.001
Gender (M)	1.35	1.03–1.77	.028	1.19	.91–1.56	.211
COB (Australia)	.80	.59–1.08	.144	–	–	–
CAP ($n = 3840$)	1.00	1.00–1.00	.488	–	–	–
LSM (kPa)[b]	1.04	1.04–1.05	<.001	1.02	1.01–1.03	<.001
Ischaemic heart disease	2.57	1.43–4.60	.002	–	–	–
Congestive cardiac failure	8.69	5.93–12.75	<.001	–	–	–
Peripheral vascular disease	6.68	4.53–9.82	<.001	–	–	–
Stroke	2.73	1.36–5.54	.005	–	–	–
Pulmonary disease	5.63	3.55–8.93	<.001	–	–	–
Connective tissue disease	2.61	1.07–6.33	.034	–	–	–
Hemiplegia	7.45	3.51–15.96	<.001	–	–	–
CKD	8.34	6.03–11.52	.001	–	–	–
T2DM—uncomplicated	1.71	1.21–2.42	.002	–	–	–
T2DM—complicated	4.67	3.19–6.84	<.001	–	–	–
Cancer—nil metastatic	2.30	1.38–3.84	.002	–	–	–
Cancer—metastatic	19.45	12.31–30.72	<.001	–	–	–
Mild liver disease	1.30	.78–2.17	.30	–	–	–
Severe liver disease	5.62	4.13–7.64	<.001	–	–	–
Charlson comorbidity index (CCI)[c]	1.44	1.41–1.49	<.001	1.32	1.27–1.38	<.001
CCI 0–2	.10	.07–.15	<.001	–	–	–
CCI 3–5	1.15	.87–1.52	.324	–	–	–
CCI 5–8	6.00	4.48–8.04	<.001	–	–	–
CCI >8	17.29	12.40–24.09	<.001	–	–	–

[a]Adjusted for age, gender, liver stiffness and Charlson comorbidity index.

[b]CCI subtracted for liver resulted in similar findings on multivariable ➔ HR 1.03 (CI 1.02–1.04).

[c]Assessed as a continuous variable.

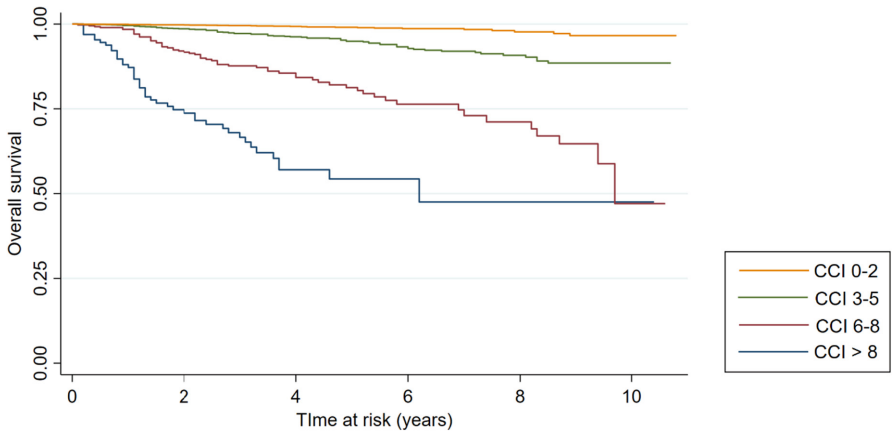

FIGURE 2 Kaplan–Meier of Charlson comorbidity index (CCI) relative to survival (orange bar = CCI 0–2, green = CCI 3–5, red = CCI 6–8, blue = CCI >8).

non-GI malignancy, sepsis, decompensated liver disease and cardiac complications. Life expectancy in our study was lower than the Australian national average which is 81.2 and 84.0 years for males and females respectively.[30]

4.2 | Comparison to other studies

Our data had some key differences from other survival studies. Notable amongst these are a lower rate of T2DM. For example,

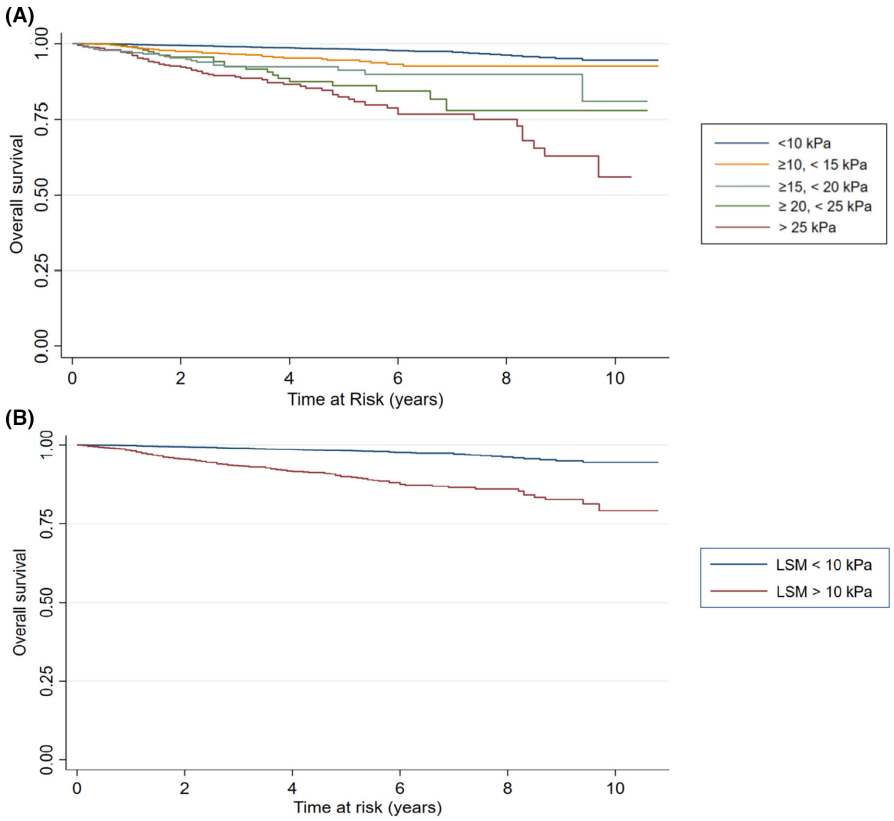

FIGURE 3 (A) Kaplan–Meier of liver stiffness measurement (LSM) (kPa) relative to survival using the rule of 5 s threshold values (blue bar = LSM < 10 kPa, orange bar = LSM ≥ 10 and < 15 kPa, teal bar = LSM ≥ 15 kPa and < 20 kPa, green bar = LSM ≥ 20 and < 25 kPa, red bar = LSM ≥ 25 kPa). (B) Kaplan–Meier of LSM (kPa) based on no cACLD (blue bar, <10 kPa), or suggestive cACLD (red bar = LSM ≥ 10 kPa).

Shili-Masmoudi et al reported 61.2% T2DM in their prospective cohort of patients who had an LSM as part of NAFLD evaluation.[19] The reason for this discordance is not clear. It is notable, however, that none of the participating health services in our study required risk-stratification prior to VCTE. VCTE, at the time of data collection, was therefore frequently used as a primary liver staging tool. As such, our data has not been enriched for people with more advanced liver disease, many of whom would have more advanced metabolic conditions, including T2DM.

Notwithstanding these differences, COD in our study was similar to longitudinal VCTE-based and histological-based survival studies, with extra-hepatic malignancy, liver disease, infection and cardiac disease borne out as most common (Table S7).[17–19,31] There data support a connection between NAFLD and extrahepatic disease. For example, NAFLD has been borne out as an independent cofactor in the development of cardiovascular disease, including subclinical carotid intimal hyperplasia, coronary artery calcification and left ventricular diastolic dysfunction.[32,33] This suggests that complex pathophysiological co-factors link NAFLD to extrahepatic complications. Whilst the pathophysiology of many NAFLD associations remains incompletely understood, systemic inflammatory cascades, both arising from and culminating in toxic hepatic lipogenesis and inflammation are likely to play a part.[2,34,35]

Our study did not show an association between CAP score and mortality. Cardoso et al in fact found paradoxical protection with increased CAP (HR = .92).[18] The CAP score varies based on comorbidities like T2DM, gender, and probe type and presently lacks discriminatory function for grading steatosis in NAFLD.[36] Histology-based longitudinal NAFLD studies had also previously failed to identify hepatic steatosis as an independent predictor of morbidity or mortality.[7] However, a recent 14.2-year Swedish nationwide longitudinal follow-up study of 4338 individuals with biopsy-proven NAFLD showed that all stages of liver disease, including simple steatosis, were associated with increased mortality when compared to a control cohort without NAFLD.[37] Taken together, steatosis severity based on CAP may be of limited utility as a single timepoint predictor of mortality, but given recent data and the natural history of fatty liver disease, the identification of steatosis without fibrosis cannot be considered completely benign.[37,38]

4.3 | Implication

VCTE has high diagnostic accuracy for determining advanced fibrosis in NAFLD,[39] notwithstanding some heterogeneity in fibrosis thresholds.[24,40–42] The wide use of VCTE for liver disease staging supports the need for studies which evaluate prognostic capacity of VCTE. Data from this study reinforce that increased liver stiffness is associated with heightened all-cause mortality and can be used to guide prognosis. We suggest that an LSM of ≥10 kPa, which is suggestive of cACLD[16] and which corresponds with ≥F3 fibrosis based on Youden cut-offs from paired biopsy data,[24] may provide a useful cut-off for early intervention and concentration of health resources. At present time, CAP appears useful as a diagnostic tool for NAFLD but does not have utility as an independent prognostic tool for mortality.

4.4 | Strengths and limitations

The strengths of this study lie in the large sample size which broadly reflects NAFLD VCTE referrals within the state of Victoria, Australia. Additionally, the use of linkage analysis has allowed for large dataset analysis, thus mitigating against ascertainment bias and loss to follow-up. Unlike many other NIT prognostication studies, the use of large datasets has allowed the calculation of and adjustment for the CCI.

There were, however, several limitations. Foremost, NAFLD inclusion criteria were predicated on information provided by the referring doctor rather than prospective, per protocol histology or ultrasound diagnosis. However, 95.6% of patients were referred for VCTE by Gastroenterologists, thus supporting the veracity of a NAFLD diagnosis. Secondly, 40.1% of CAP scores fell below the high sensitivity S ≥ 1 cut-off of 274 db/m reported by Eddowes et al.[24] However, CAP performance varies widely in detecting and grading steatosis.[36] For example, Park et al. compared VCTE performance to liver histopathology in consecutive patients with suspected NAFLD and found that a CAP threshold of 261 db/m had a sensitivity of 71.8% and negative predictive value of 23.1% for detecting steatosis.[43] Of note, median CAP score in the Park et al. study was comparable to that of our cohort (293 db/m vs. 299 db/m, respectively), and almost all patients, 90.4%, had evidence of ≥S1 steatosis on liver histopathology.[43] At present, there remains a lack of consensus regarding optimal CAP cut off values, thus limiting the use of CAP as a diagnostic or study inclusion criterion for NAFLD.[36]

Another limitation is that most comorbidity and COD data were not prospectively collected. This may have led to incomplete data ascertainment and interpretation. For example, sepsis, which was the primary attributable COD in many patients, may have been reclassified as a precipitant rather than primary COD in a prospectively evaluated cohort. We also note that comorbidity rates were higher in people who had >1 day admissions. This may have been a function of more complex patients requiring longer admissions, but equally may reflect a lower propensity to code healthcare information for patient admissions of <1 day. An additional corollary of the large cohort retrospective design is that liver function test biochemistry was not available for many patients. As such, we could not test composite associations like the FAST score on mortality, nor could we compare other NITs relative to VCTE.[44] We also acknowledge that our data only reflect single timepoint VCTE, and changes over time would certainly be of interest for future research. Lastly, our data did not have an international validation cohort, which may reduce generalisability. However, we intentionally captured a large cohort across a broad geographic catchment to try and mitigate subpopulation and demographic selection biases. Acknowledging these limitations, we show that VCTE independently predicts overall mortality in a large Australian cohort. This contention is supported by robust research methodology, including the use of a large validated dataset, standardised clinical endpoints drawn from state-wide data linkage, and adjustment for cofounding data, particularly through this use of the CCI.

5 | CONCLUSION

Non-alcoholic fatty liver disease is a highly prevalent, emerging health condition with multisystem implications. Non-invasive liver stiffness tools have increasing utility and reliability in staging liver disease, and furthermore, in prognosticating global health outcomes. We show that liver stiffness, in addition to age and Charlson Co-morbidity index, is independently associated with mortality in a large NAFLD cohort. VCTE may therefore be recognised as an additional important prognostic marker of all-cause mortality in people with NAFLD.

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CONFLICT OF INTEREST

None of the authors has a disclosed conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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